

A widespread morphological antipredator mechanism reduces the sensitivity to pesticides and increases the susceptibility to warming



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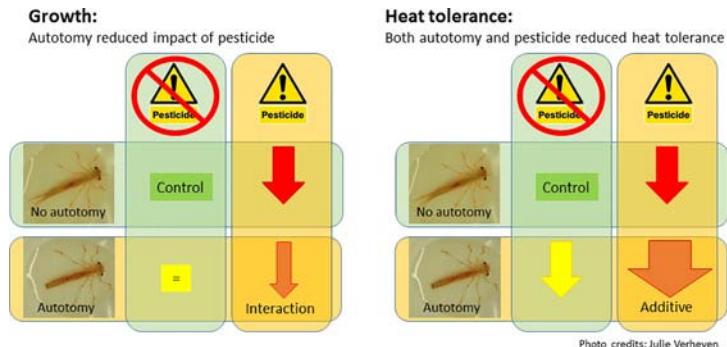
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HIGHLIGHTS

- Pollution and predation are omnipresent interacting stressors.
- The antipredator mechanism autotomy reduced the heat tolerance in damselfly larvae.
- Chlorpyrifos reduced the growth rate and heat tolerance.
- The impact of the pesticide on growth was smaller in autotomized larvae.
- Antipredator mechanisms can shape the sensitivity to pollutants.

GRAPHICAL ABSTRACT



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ABSTRACT

Pollution and predation are two omnipresent stressors in aquatic systems that can interact in multiple ways, thereby challenging accurate assessment of the effects of pollutants in natural systems. Despite the widespread occurrence of morphological antipredator mechanisms, no studies have tested how these can affect the sensitivity of prey to pesticides. Sensitivity to pesticides is typically measured via reductions in growth rates and survival, but also reductions in heat tolerance are to be expected and are becoming increasingly important in a warming world. We investigated how autotomy, a widespread morphological antipredator mechanism where animals sacrifice a body part (here the caudal lamellae) to escape when attacked by a predator, modified the sensitivity to the insecticide chlorpyrifos in larvae of the damselfly *Coenagrion puella*. Exposure to chlorpyrifos reduced the growth rate and heat tolerance (measured as CTmax). A key finding was that the pesticide had a greater impact on growth rates of intact animals, i.e. those that retained their lamellae. This reduced sensitivity to chlorpyrifos in animals without lamellae can be explained by the reduced outer surface area which is expected to result in a lower uptake of the pesticide. Larvae that underwent autotomy exhibited a lower heat tolerance, which may also be explained by the reduced surface area and the associated reduction in oxygen uptake. There is a wide diversity of morphological antipredator mechanisms, suggesting that there will be more examples where these mechanisms affect the vulnerability to pollutants. Given the importance of pollution and predation as structuring forces in aquatic food webs, exploring the potential interactions between morphological antipredator mechanisms and sensitivity to pollutants will be crucial for risk assessment of pollutants in aquatic systems.

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1. Introduction

Pesticides (Liess et al., 2008) and predation (Kerfoot and Sih, 1987) are omnipresent stressors in aquatic ecosystems that may interact in multiple ways. Recent studies show that exposure to predation risk makes pesticides more toxic (e.g. Relyea and Mills, 2001; Campero et al., 2007), likely through energy limitation (Sokolova, 2013; Liess et al., 2016), and that pesticide exposure impairs antipredator mechanisms (e.g. Janssens and Stoks, 2012; Shuman-Goodier and Propper, 2016) and predator recognition (e.g. Polo-Cavia et al., 2016). Yet, such interactions are not found consistently (e.g. Jansen et al., 2011) and a recent study showed that exposure to the herbicide glyphosate actually increased the chemical antipredator defence of toad larvae (*Bufo bufo*) (Bokony et al., 2017). Morphological antipredator mechanisms are widespread (Kerfoot and Sih, 1987; Barbosa and Castellanos, 2005) and are therefore likely to also be displayed in polluted habitats. However, so far no studies directly tested how morphological antipredator mechanisms can affect the sensitivity to pesticides.

An important morphological antipredator mechanism in both vertebrates and invertebrates (McVean, 1975; Fleming et al., 2007) is autotomy, the amputation of a body part at a breakage plane to escape when caught by a predator (Fleming et al., 2007). There are many delayed costs associated with autotomy; for example, a lower escape speed (e.g. Gyssels and Stoks, 2005), a reduced balance during locomotion (Hessell et al., 2017) and reduced energy stores because of re-growing a body part (Diaz-Guisado et al., 2006), resulting in an increased sensitivity to predation in the future (e.g. Stoks, 1998a). In damselfly larvae, autotomy has been shown to not affect resting metabolic rate (Sesterhenn et al., 2013), but to lower antioxidant defence and immune function (Slos et al., 2009). Whether autotomy may influence the sensitivity to pesticides is an unexplored, yet exciting pathway in the interaction between predation and pesticides. If autotomy reduces the outer surface area, then the impact of pollutants may be reduced. Indeed, in many aquatic organisms the majority of the uptake of pollutants occurs through the surface and it has been shown that aquatic arthropods with a larger outer surface area and external gills are more sensitive to pesticides due to a higher uptake, hence higher internal accumulation of the toxicant (e.g. Rubach et al., 2012; Wiberg-Larsen et al., 2016).

Sensitivity to pesticides is typically measured via reductions in growth rates and survival, but also reductions in heat tolerance are to be expected and are becoming increasingly important in a warming world. Heat tolerance is set by oxygen limitation, particularly a mismatch between the capacity for oxygen supply to body tissues and the rate at which oxygen is required (Ern et al., 2015; Verberk et al., 2016a). In ectotherms, the demand for oxygen increases with temperature, potentially outpacing the rate at which oxygen can be supplied (Pörtner, 2010; Verberk et al., 2011). Pesticide exposure is expected to reduce heat tolerance (see e.g. Op de Beeck et al., 2017) as pesticide exposure increases oxygen demand, making a mismatch between the capacity for oxygen supply and the rate at which oxygen is required more likely (Sokolova and Lannig, 2008; Noyes and Lema, 2015). This general phenomenon of contaminants reducing an organism's ability to cope with increased temperatures has been named the "contaminant-induced climate sensitivity concept" (Sokolova and Lannig, 2008; Noyes and Lema, 2015). How antipredator mechanisms may shape the ability to cope with warming is unexplored. If heat tolerance is governed by a mismatch between the capacity for oxygen supply and the rate at which oxygen is required, then gill autotomy will decrease the heat tolerance because of a reduction in the capacity for oxygen uptake. This would be an important overlooked mechanism how predators may shape the ability of prey populations to deal with warming. Given the potential of pesticide exposure to increase oxygen demand and of gill autotomy to reduce oxygen uptake, their combination may especially reduce heat tolerance.

Pesticides and heat waves are important stressors in ponds as the inhabitants cannot escape exposure (Stoks et al., 2015; Hassall and

Thompson, 2008). Moreover, most aquatic insects employ under-water gas exchange, which also involves a diffusive step. This makes them especially relevant to study in the context of heat tolerance because the low solubility and diffusivity of oxygen challenges diffusive uptake of oxygen (Verberk and Atkinson, 2013). Indeed, aquatic insects that relied on under-water gas exchange, including damselfly larvae (Verberk and Calosi, 2012), were more prone to oxygen-limited heat tolerance (Verberk and Bilton, 2013), and will likely show reduced heat tolerance when also exposed to pesticides.

In this study, we tested the single and combined effects of pesticide exposure and autotomy on survival, growth rate and heat tolerance (quantified as CTmax) on larval damselflies. We chose the pesticide chlorpyrifos. This model pesticide for the class of organophosphates is one of the most frequently used insecticides worldwide (Eaton et al., 2008) and a priority pollutant in the European Water Framework Directive (2000/60/EC). As study species we use *Coenagrion puella* a common damselfly species in Europe for which we have shown negative effects of chlorpyrifos on food intake (Tüzün et al., 2015) and growth rate (Dinh Van et al., 2016). Growth rate is an indicator for the adult mass, hence reproductive potential in damselflies (Stoks and Cordoba-Aguilar, 2012). CTmax is considered a useful proxy for the capacity of individuals to withstand extreme heat (Huey et al., 2012). The ability of CTmax to predict field patterns in the distribution of species and how these are affected by warming indicates CTmax is informative for how organisms can cope with climate change (e.g. Calosi et al., 2010; Verberk et al., 2016b; Birkett et al., 2017). We chose to study damselfly larvae as these often autotomize their caudal lamellae, with frequencies of autotomy in natural populations going from 30% to 90% (Stoks, 1998a; Witt et al., 2013). Furthermore, the benefits and costs of autotomy have been well studied in these animals (e.g. Stoks, 1998a; Slos et al., 2009). We expect the pesticide to reduce both the growth rate (e.g. Janssens and Stoks, 2013) and heat tolerance (e.g. Op de Beeck et al., 2017), because of energy limitations and a disturbance of the oxygen metabolism. Due to the reduction of the surface area, autotomy is expected to lower the oxygen uptake and therefore reduce the heat tolerance (Verberk and Bilton, 2011), but at the same time the lower pesticide uptake and accumulation will reduce the sensitivity to the pesticide. We therefore expect the effects of the pesticide and autotomy to interact with autotomy conferring the benefit of reduced pesticide sensitivity.

2. Methods

We collected larvae of the damselfly *Coenagrion puella* in a fishless pond in Heverlee (Belgium). Large dragonfly larvae (*Anax* and *Aeshna* species) are top predators in these water bodies (Stoks et al., 2003). We only collected damselfly larvae with three intact, not regenerated lamellae. This pond is not surrounded by agricultural fields making it less likely larvae experienced pesticide exposure (Coors et al., 2009). Because damselfly larvae are cannibalistic (De Block and Stoks, 2004), individuals were kept in the laboratory in separate 100 mL plastic vials under standard conditions of light (14:10 L:D), temperature (22 °C) and food (ad libitum *Artemia* nauplii) for at least four weeks.

2.1. Experimental setup

To test for the effects of autotomy and pesticide exposure and their interaction on growth and heat tolerance, we set up a full factorial 2 × 4 experiment combining two autotomy treatments (control or autotomized larvae) with four pesticide treatments (0, 0.75, 1.0, 1.5 µg/l chlorpyrifos), giving a total of eight treatment combinations. We used penultimate instar (F_1) larvae for the experiment and exposed the animals to the treatments for seven days. We quantified mortality and growth rate across the 7-day exposure period and CTmax at the end of the 7-day exposure period. The mass of the damselfly larvae at the beginning of the exposure experiment varied between 15.65 and

33.28 mg with a mean (± 1 S.E.) of 24.04 ± 6.92 mg. We started 24 replicates per treatment combination (total N = 192).

One day after moulting into the penultimate instar, the larvae were transferred to 100 ml glass vials filled with 50 ml of medium (solvent control or chlorpyrifos) for the exposure treatment. During a chronic 7 day exposure period, larvae were fed *Artemia* nauplii ad libitum daily. The chlorpyrifos concentrations (0.75, 1.0 and 1.5 µg/l) were selected based on previous experiments on damselfly larvae, where they induced sublethal effects on growth, behaviour and physiology without causing mortality (Dinh Van et al., 2014a; Janssens et al., 2017). The chosen chlorpyrifos concentrations are high, yet within the range reported in edge-to-field water bodies (Schulz, 2004). To prepare the chlorpyrifos solutions for the exposure we first prepared a stock solution containing 1 mg/ml of chlorpyrifos dissolved in ethanol which was kept in the dark at 4 °C. In a second step, we diluted this stock solution 100 times to obtain a concentration of 10 µg/ml chlorpyrifos. The latter solution was prepared daily to prepare the actual chlorpyrifos exposure solutions (in aerated tap water) used in the experiment. In the control condition we added the same amount of ethanol to the aerated tap water as in the condition with the highest chlorpyrifos concentration (1.5 µl/l). Previous studies on damselfly larvae showed this ethanol concentration not to affect behaviour and growth (e.g. Janssens et al., 2017). We performed a static renewal experiment whereby the medium was completely renewed every day. A mixed sample of ten experimental cups per concentration was analyzed by the laboratory of Phytopharmacy (University of Ghent) using liquid chromatography combined with tandem mass spectrometry. The measured initial concentrations were 0.64 (nominal 0.75), 0.94 (nominal 1.0) and 1.49 (nominal 1.5) µg/l chlorpyrifos; after 24 h (just before the renewal of the medium) the concentrations dropped to 0.41, 0.61 and 0.91 µg/l.

For the autotomy treatment, we haphazardly divided larvae into two groups and removed all three lamellae from one group of larvae per pesticide exposure combination by gently pulling them with tweezers until the animals autotomized these appendages at the specialized breaking joints (as in Stoks, 1999). Damselfly larvae often lose all three lamellae simultaneously in the field (Stoks, 1998b). The other half of the larvae underwent a sham operation without removing the lamellae.

2.2. Response variables

We monitored survival daily during the exposure period. We quantified growth rate as the increase in body mass per day; due to some mortality (see results) we were able to do so in 19–23 replicates per treatment combination (total N = 166). Animals were blotted dry using tissue paper and weighed at the beginning and at the end of the 7-day exposure period using an electronic balance (0.01 mg precision) and growth rate was calculated as relative growth rate using the formula $(\ln_{\text{end mass}} - \ln_{\text{begin mass}}) / 7$. This measure of growth rate is preferred as it is independent of the initial mass of the damselfly larvae (e.g. Stoks et al., 2003).

Immediately after the exposure period (on day 7), we quantified the critical thermal maximum (CTmax) as a proxy for the heat tolerance following the methodology described in Op de Beeck et al. (2017). For logistic reasons we tested CTmax for a randomly chosen subset of the larvae (13–17 per treatment combination, total N = 120). Shortly, the larvae were placed individually in plastic vials filled with aged tap water. Those vials were placed in a temperature controlled water bath filled with water with an initial temperature of 22 °C. An aquarium heater heated up the water with a constant rate of 0.30 °C per minute. This heating rate falls within the range used to study CTmax in freshwater insects (e.g. Verberk and Bilton, 2011; Dallas and Rivers-Moore, 2012). The temperature at which the larvae entered a heat coma, i.e. became moribund and no longer showed any body movements, was defined as the CTmax (Verberk and Calosi, 2012; Verberk and Bilton, 2013). After the CTmax test, larvae were placed again at 22 °C; all larvae recovered. CTmax trials were done on different, consecutive days, reflecting

the fact that not all larvae reached the penultimate instar at the same day and hence the period during which they were exposed to pesticides also ended at different, consecutive days. We included the trial day (the day at which the CTmax was measured) in the statistical analyses. Per trial day we tested 17–18 larvae (2–3 larvae per treatment combination).

2.3. Statistical analyses

Survival was analyzed using a general linear model (GLM) with a binomial error structure with autotomy, the pesticide treatment and their interaction as fixed effects. We tested for an effect of autotomy, the pesticide treatment and their interactions on growth rate and CTmax using separate two-way GLMs with a normal error structure. For growth rate, we initially included initial body mass as a covariate but as it was not significant ($p = 0.21$) it was removed from the final model. Note this also confirmed our estimate of growth rate was size-independent. For CTmax we initially included trial day (day of measurement) as a random effect and body mass as covariate, but since these had no effect (both $p > 0.25$), we removed them from the final model. None of the covariates interacted with the autotomy or pesticide treatment. In case of an interaction between the pesticide and autotomy treatments, we tested the main effect of pesticide in additional GLMs separately for damselfly larvae with and without lamellae. When the main effect of pesticide was significant in these additional GLMs, we used follow-up Tukey post hoc tests to investigate which of the different pesticide concentrations differed significantly from one another. We confirmed normality using Shapiro Wilk tests (growth: $p = 0.15$; CTmax: $p = 0.37$) and homogeneity of variances using Levene tests (growth: $p = 0.18$; CTmax: $p = 0.49$). All tests were done in Statistica v13 (Statsoft Inc., OK, USA).

3. Results

Survival was high overall (on average 86.1%) and did not differ between the treatments (all $p > 0.35$).

There was a significant interaction between the pesticide and autotomy treatment for growth rate ($F_{3, 158} = 2.70$; $p = 0.048$; Fig. 1). The additional GLMs per autotomy treatment indicated that the pesticide significantly reduced the growth rate both in larvae with lamellae (main effect pesticide: $F_{3, 82} = 7.23$; $p = 0.0023$) and in larvae without lamellae (main effect pesticide: $F_{3, 76} = 5.24$; $p = 0.0024$). However, the follow-up Tukey tests showed that the concentration at which the growth reduction occurred depended on the autotomy treatment: damselfly larvae with lamellae showed considerably reduced growth rates at the intermediate (1 µg/l: Tukey $p = 0.012$) and high (1.5 µg/l: Tukey $p < 0.001$) chlorpyrifos concentrations compared to the control condition. Moreover, in damselfly larvae with lamellae, there was also a significant difference in growth rate between the two highest chlorpyrifos concentrations, with the growth being further reduced at 1.5 µg/l (Tukey $p = 0.029$). In contrast, in larvae that autotomized their lamellae, growth was reduced only at the highest pesticide concentration (1.5 µg/l: Tukey $p = 0.018$).

There was no significant interaction between the pesticide and the autotomy treatment for CTmax ($F_{3, 106} = 1.00$; $p = 0.39$), although there was a trend that in the larvae with lamellae chlorpyrifos reduced CTmax already at a lower concentration compared to the animals without lamellae (Fig. 2). In general, CTmax was lower in autotomized larvae (main effect autotomy: $F_{1, 106} = 21.60$; $p < 0.001$) and in animals exposed to chlorpyrifos (main effect pesticide: $F_{3, 106} = 17.70$; $p < 0.001$).

4. Discussion

We tested whether a widespread morphological antipredator mechanism such as autotomy may shape the sensitivity of prey organisms to pesticides and to heat. As expected, gill autotomy resulted in damselfly

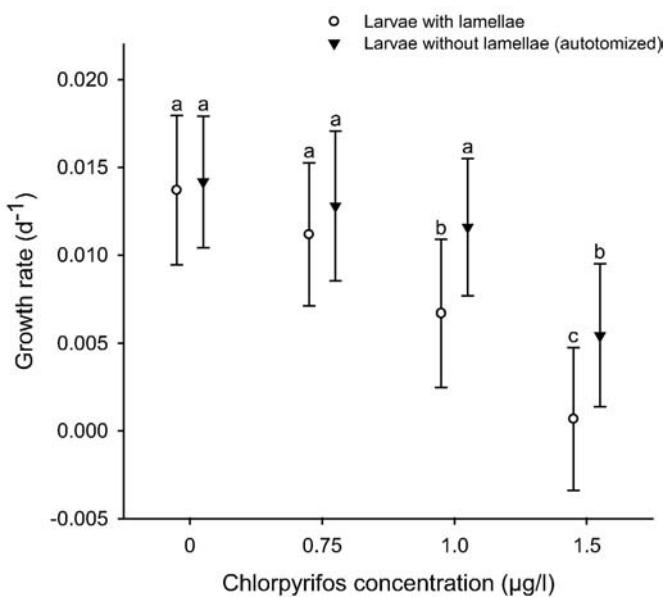


Fig. 1. Mean growth rate (with 95% confidence intervals) of *Coenagrion puella* damselfly larvae as a function of pesticide exposure and autotomy. Different letter codes indicate significant differences between the treatments based on Tukey post hoc tests.

larvae being less sensitive to chlorpyrifos (for growth rate), but more sensitive to warming (reduced CTmax). Furthermore, our results support the “contaminant-induced climate sensitivity concept” (Sokolova and Lannig, 2008; Noyes and Lema, 2015) by showing that larvae exposed to chlorpyrifos had a reduced heat tolerance.

The pesticide caused no mortality but resulted in a reduced growth rate (on average –54%) and a decrease in heat tolerance (CTmax) (on average –0.95 °C). A lower growth rate during chlorpyrifos exposure

has been shown in many aquatic species (e.g. tadpoles (Widder and Bidwell, 2006), fish (Huynh and Nugget, 2012)) including damselfly larvae (e.g. Dinh Van et al., 2014a; Janssens et al., 2017). This chlorpyrifos-induced growth reduction can be explained by a combination of a decrease in food consumption (e.g. Widder and Bidwell, 2006; Dinh Van et al., 2014b) and an increased investment in costly defence processes against the pesticide. Examples of the latter are increased levels of the stress protein Hsp70 (Scheil et al., 2010; Janssens and Stoks, 2013), and increased activity of the detoxification enzyme Glutathione-S-transferase, GST (Cinzia et al., 2006; Janssens and Stoks, 2013).

The observed pesticide-induced reduction in heat tolerance matches the general pattern in pesticides (Noyes and Lema, 2015), including chlorpyrifos (e.g. Patra et al., 2007; Op de Beeck et al., 2017). Stressors such as pesticides may result in a reduced heat tolerance by causing oxygen demand to be higher than oxygen supply (Verberk and Bilton, 2011, 2013). Individuals exposed to pesticides indeed often increase their aerobic metabolism, and thus oxygen consumption. For example, Bednarska and Kaszowska (2014) showed in ground beetles exposed to chlorpyrifos an increase in respiration rate. Increases in respiration rate in response to moderate stress are assumed to be general and to be driven, amongst others, by the increased energy demand to activate mechanisms for protection and damage repair (Sokolova, 2013). Whether pesticide exposure also reduces the internal supply of oxygen to body tissues is unknown. Other processes that reduce the available energy for the organism and thereby affect the aerobic performance may also contribute to the pesticide-induced lower CTmax (reviewed in Noyes and Lema, 2015). For example, exposure to chlorpyrifos has been shown to lower food intake in the study species (Tüzün et al., 2015), and to lower food intake and energy stores in other damselfly species (e.g. Dinh Van et al., 2014a, 2014b; Arambourou and Stoks, 2015; Janssens et al., 2017).

As expected, the heat tolerance was lower in animals without lamellae. The caudal lamellae of damselfly larvae function as gills for the uptake of oxygen, and this function gets more important with increasing temperature (Eriksen, 1986). Given the importance of oxygen supply for the heat tolerance in damselfly larvae (Verberk and Calosi, 2012), this may explain the lower CTmax in autotomized animals. Similarly, Eriksen (1986) showed in another damselfly species, that larvae with lamellae could better survive long-term exposure to higher water temperatures than larvae without lamellae. A reduction in heat tolerance has been a largely ignored cost of autotomy (for example, not included in the overview given by Fleming et al., 2007) that likely will become more important in a warming world. Note that autotomy did not reduce the growth rate, which may be explained by the presence of ad libitum food (Starostova et al., 2017).

A key finding of our study was that the negative impact of the pesticide for growth was greater in intact, non-autotomized animals. Indeed, the lowest observed effect concentration (LOEC) for growth rate was higher for autotomized larvae (1.5 µg/l) compared to larvae without lamellae (1 µg/l). Since chlorpyrifos is a hydrophobic compound it is expected to sorb onto the outer surface of the larvae, making surface area to volume relationships important determinants of chlorpyrifos accumulation (Buchwalter et al., 2004; Rubach et al., 2012). Given the surface of lamellae can make up a considerable amount relative to the total outer surface area of damselfly larvae (e.g. up to 68% in the damselfly *Lestes disjunctus*, Eriksen, 1986), lamellae are not only important for oxygen uptake (Eriksen, 1986), but likely also for the uptake of pollutants. Hence, animals with lamellae likely had a higher internal chlorpyrifos accumulation. Similarly, Phillips et al. (2002) showed an increased sensitivity to chlorpyrifos in juvenile walleyes at the moment they developed gill filaments and more sophisticated respiratory systems and also hypothesized this to be the result of increased uptake of the pesticide. As damselflies cannot actively remove their lamellae they cannot exploit autotomy to reduce their sensitivity. Moreover, lamellae autotomy comes with costs such as increased susceptibility to predation (see introduction). Nevertheless, exposure to chlorpyrifos has been shown

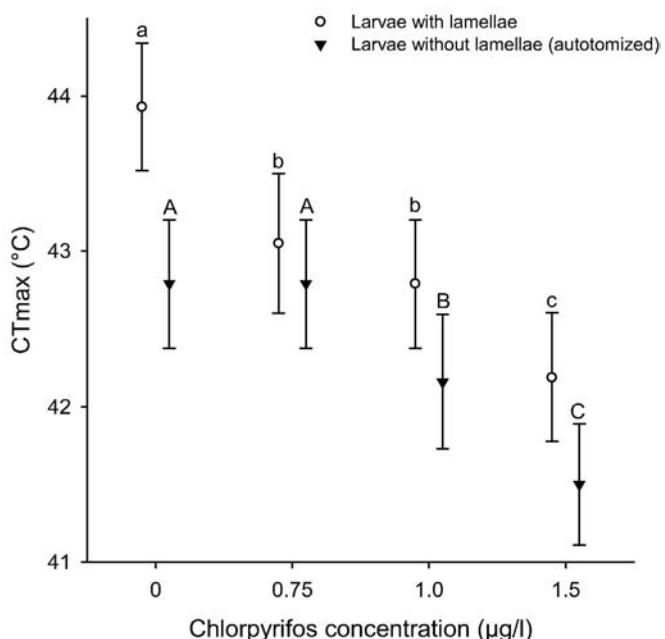


Fig. 2. Mean upper thermal tolerance (measured as critical thermal maximum, CTmax) (with 95% confidence intervals) of *Coenagrion puella* damselfly larvae as a function of pesticide exposure and autotomy. Different letter codes indicate significant differences between the treatments based on Tukey post hoc tests with lower case letter codes illustrating the effect of the pesticide for larvae with lamellae and capital letters illustrating the effect of the pesticide for larvae without lamellae.

to increase the degree of autotomy in larvae from northern populations in another coenagrionid damselfly when tested under mild warming (Op de Beeck et al., 2018). Noteworthy, in *Tubifex* worms the self-amputation of the contaminated, posterior body regions has been described as an active strategy to excrete contaminants (Paris-Palacios et al., 2010).

5. Implications for risk assessment of pesticides

Our findings are important to improve risk assessment of pesticides and illustrate the relevance of integrating freshwater ecology and ecotoxicology (Gessner and Tili, 2016). Coping with extreme temperatures will become increasingly important under climate change, since heat waves will become more intense, more frequent and of longer duration (Christidis et al., 2015). Our data demonstrate a clear interaction between a pollutant and an antipredator mechanism in terms of growth rate and show that heat tolerance can be a more sensitive biomarker than the more typically used sublethal biomarker growth rate. Indeed, the non-autotomized larvae already showed a decrease in CTmax at a chlorpyrifos concentration of 0.75 µg/l, while the growth reduction was only observed at a concentration of 1 µg/l.

An increasing number of studies demonstrated that predation risk can make pesticides more toxic (e.g. Relyea and Mills, 2001; Campero et al., 2007) and that pesticide exposure may impair antipredator mechanisms, such as predator escape responses (Janssens and Stoks, 2012). To our knowledge this study is the first to show an antipredator mechanism reducing the sensitivity to a pollutant. The beneficial effect of autotomy by reducing pesticide sensitivity is expected to be general as it is most likely operating through the reduction of uptake of the pesticide because of a reduced surface area. This would mean that in natural damselfly populations where autotomy frequently occurs (Stoks, 1998a; Witt et al., 2013), animals could be less sensitive to pesticides than predicted based on laboratory studies. While the mechanism is likely common amongst taxonomic groups, the predicted effects can vary. For example, tadpoles typically increase their tail surface area when exposed to predatory dragonfly larvae (e.g. Van Buskirk and McCollum, 2000), which by increasing the outer surface area may make them more vulnerable to pollutants such as chlorpyrifos. Other examples of morphological antipredator responses that will increase the outer surface area are the change to a deep-bodied morph in carps in response to gape-limited predators (e.g. Domenici et al. 2008) and the formation of neck teeth in water fleas in response to predator kairomones (e.g. Hanazato and Dodson, 1995). Given the importance of pollution, predation and warming as structuring forces in aquatic food webs (Kerfoot and Sih, 1987; Thomas et al., 2004; Halstead et al., 2014) exploring these potential interactions between antipredator mechanisms, their associated costs, and sensitivity to pollutants will be crucial to improve the risk assessment of pollutants in aquatic systems under climate change.

Conflict of interest

The authors have no conflict of interest to declare.

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References

- Arambourov, H., Stoks, R., 2015. Combined effects of larval exposure to a heat wave and chlorpyrifos in northern and southern populations of the damselfly *Ischnura elegans*. *Chemosphere* 128, 148–154.
- Barbosa, P., Castellanos, I., 2005. *Ecology of Predator-prey Interactions*. Oxford University Press, New York.
- Bednarska, A.J., Kaszowska, J., 2014. Combined effect of nickel and chlorpyrifos on the ground beetle *Presostichus oblonopunctatus*. *Ecotoxicol. Environ. Saf.* 108, 242–248.
- Birkett, A.J., Blackburn, G.A., Menéndez, R., 2017. Linking species thermal tolerance to elevational range shifts in upland dung beetles. *Ecography* <https://doi.org/10.1111/ecog.03458>.
- Bokony, V., Miko, Z., Moricz, A.M., Kruszelyi, D., Hettyey, A., 2017. Chronic exposure to a glyphosate-based herbicide makes toad larvae more toxic. *Proc. R. Soc. B* 284, 20170493.
- Buchwalter, D.B., Sandahl, J.F., Jenkins, J.J., Curtis, L.R., 2004. Roles of uptake, biotransformation and target site sensitivity in determining the differential toxicity of chlorpyrifos to second to fourth instar *Chironomus riparius* (Meigen). *Aquat. Toxicol.* 66, 149–157.
- Calosi, P., Bilton, D.T., Spicer, J.I., Votier, S.C., Atfield, A., 2010. What determines a species' geographical range? Thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). *J. Anim. Ecol.* 79, 194–204.
- Campero, M., Slos, S., Ollevier, F., Stoks, R., 2007. Sublethal pesticide concentrations and predation jointly shape life history: behavioral and physiological mechanisms. *Ecol. Appl.* 17, 2111–2122.
- Christidis, N., Jones, G.S., Stott, P.A., 2015. Dramatically increasing chance of extremely hot summers since the 2003 European heat wave. *Nat. Clim. Chang.* 5, 46–50.
- Cinzia, A., Baldracchini, F., Piazzioli, A., Frosini, R., Talesa, V., Elvio, G., 2006. Activity changes of glyoxalase system enzymes and glutathione-S-transferase in the bivalve mollusk *Scapharca inaequivalvis* exposed to the organophosphate chlorpyrifos. *Pestic. Biochem. Physiol.* 86, 72–77.
- Coors, A., Vanoverbeke, J., De Bie, T., De Meester, L., 2009. Land use, genetic diversity and toxicant tolerance in natural populations of *Daphnia magna*. *Aquat. Toxicol.* 95, 71–79.
- Dallas, H.F., Rivers-Moore, N.A., 2012. Critical thermal maxima of aquatic macroinvertebrates: towards identifying bioindicators of thermal alteration. *Hydrobiologia* 679, 61e76.
- De Block, M., Stoks, R., 2004. Cannibalism-mediated life history plasticity to combined time and food stress. *Oikos* 106, 587–597.
- Díaz-Guisado, D., Daymer, C.F., Brokordt, K.B., Lawrence, J.M., 2006. Autotomy reduces feeding, energy storage and growth of the sea star *Stichaster striatus*. *J. Exp. Mar. Biol. Ecol.* 338, 73–80.
- Dinh Van, K., Janssens, L., Debecker, S., Stoks, R., 2014a. Temperature- and latitude-specific individual growth rates shape the vulnerability of damselfly larvae to a widespread pesticide. *J. Appl. Ecol.* 51, 919–928.
- Dinh Van, K., Janssens, L., Debecker, S., Stoks, R., 2014b. Warming increases chlorpyrifos effects on predator but not anti-predator behaviours. *Aquat. Toxicol.* 152, 215–221.
- Dinh Van, K., Janssens, L., Stoks, R., 2016. Exposure to a heat wave under food limitation makes an agricultural insecticide lethal: a mechanistic laboratory experiment. *Glob. Chang. Biol.* 22, 3361–3372.
- Domenici, P., Turesson, H., Brodersen, J., Brönmark, C., 2008. Predator-induced morphology enhances escape locomotion in crucian carp. *Proc. R. Soc. Lond. B* 275, 185–201.
- Eaton, D.L., Daroff, R.B., Autrup, H., Bridges, J., Buffler, P., Costa, L.G., Coyle, J., McKhann, G., Mobley, W.C., Nadel, L., 2008. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. *Crit. Rev. Toxicol.* 38, 1–125.
- Eriksen, C.H., 1986. Respiratory roles of caudal lamellae (gills) in a lepidopteran damselfly (Odonata: Zygoptera). *J. N. Am. Benthol. Soc.* 5, 16–27.
- Ern, R., Huong, D.T.T., Phuong, N.T., Madsen, P.T., Wang, T., Bayley, M., 2015. Some like it hot: thermal tolerance and oxygen supply capacity in two eurythermal crustaceans. *Sci. Rep.* 5, 10743.
- Fleming, P.A., Muller, D., Bateman, P.W., 2007. Leave it all behind: a taxonomic perspective of autotomy in invertebrates. *Biol. Rev.* 82, 481–510.
- Gessner, M.O., Tili, A., 2016. Fostering integration of freshwater ecology with ecotoxicology. *Freshw. Biol.* 61, 1991–2001.
- Gyssels, F.G.M., Stoks, R., 2005. Threat-sensitive responses to predator attacks in a damselfly. *Ethology* 111, 411–423.
- Halstead, N.T., McMahon, R.A., Johnson, S.A., Raffel, T.R., Romansic, J.M., Crumrine, P.W., Rohr, J.R., 2014. Community ecology theory predicts the effects of agrochemical mixtures on aquatic biodiversity and ecosystem properties. *Ecol. Lett.* 17, 932–941.
- Hanazato, T., Dodson, S.I., 1995. Synergistic effects of low oxygen concentration, predator kairomone and a pesticide on the cladoceran *Daphnia pulex*. *Limnol. Oceanogr.* 40, 700–709.
- Hassall, C., Thompson, D.J., 2008. The effects of environmental warming on Odonata: a review. *Int. J. Odonatol.* 11, 131–153.
- Hessell, A.L., Ryerson, W.G., Whitenack, L.B., 2017. Doing without: jump performance after tail autotomy in three species of plethodontid salamanders. *Herpetologica* 73, 100–104.
- Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A., Jess, M., Williams, S.E., 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philos. Trans. R. Soc. Lond. B* 367, 1665–1679.
- Huynh, H., Nugeoda, D., 2012. Effects of chlorpyrifos on growth and food utilization in Australian catfish, *Tandanus tandanus*. *Bull. Environ. Contam. Toxicol.* 88, 25–29.
- Jansen, M., De Meester, L., CieLEN, A., Buser, C.C., Stoks, R., 2011. The interplay of past and current stress exposure on the water flea *Daphnia*. *Funct. Ecol.* 25, 974–982.
- Janssens, L., Stoks, R., 2012. How does a pesticide pulse increase vulnerability to predation? Combined effects on behavioral traits and escape swimming. *Aquat. Toxicol.* 110–111, 91–98.

- Janssens, L., Stoks, R., 2013. Exposure to a widespread non-pathogenic bacterium magnifies sublethal pesticide effects in the damselfly *Enallagma cyathigerum*: from the suborganismal level to fitness-related traits. *Environ. Pollut.* 177, 143–149.
- Janssens, L., Op de Beeck, L., Stoks, R., 2017. Stoichiometric responses to an agricultural pesticide are modified by predator cues. *Environ. Sci. Technol.* 51, 581–588.
- Kerfoot, W.C., Sih, A., 1987. In: Kerfoot, W.C., Sih, A. (Eds.), *Predation, Direct and Indirect Impacts on Aquatic Communities*. Hannover University Press, New England.
- Liess, M., Schäfer, R.B., Schriever, C.A., 2008. The footprint of pesticide stress in communities – species traits reveal community effects of toxicants. *Sci. Total Environ.* 406, 484–490.
- Liess, M., Foit, K., Knillmann, S., Schäfer, R.B., Liess, H.-D., 2016. Predicting the synergy of multiple stress effects. *Sci. Rep.* 6, 32965.
- McVean, A., 1975. Mini-review: autotomy. *Comp. Biochem. Physiol. A* 51, 497–505.
- Noyes, P.D., Lema, S.C., 2015. Forecasting the impacts of chemical pollution and climate change interactions on the health of wildlife. *Curr. Zool.* 61, 669–689.
- Op de Beeck, L., Verheyen, J., Stoks, R., 2017. Integrating both interaction pathways between warming and pesticide exposure on upper thermal tolerance in high- and low- latitude populations of an aquatic insect. *Environ. Pollut.* 224, 714–721.
- Op de Beeck, L., Verheyen, J., Stoks, R., 2018. Competition magnifies the impact of a pesticide in a warming world by reducing heat tolerance and increasing autotomy. *Environ. Pollut.* 233, 226–234.
- Paris-Palacios, S., Mosleh, Y.Y., Almohamad, M., Delahaut, L., Conrad, A., Arnoult, F., Biagianti-Risbourg, S., 2010. Toxic effects and bioaccumulation of the herbicide isoproturon in *Tubifex tubifex* (Oligochaeta, Tubificidae): a study of significance of autotomy and its utility as a biomarker. *Aquat. Toxicol.* 98, 8–14.
- Patra, R.W., Chapman, J.C., Lim, R.P., Gehrk, P.C., 2007. The effects of three organic chemicals on the upper thermal tolerances of four freshwater fishes. *Environ. Toxicol. Chem.* 26, 1454–1459.
- Phillips, T.A., Wu, J., Summerfelt, R.C., Atchison, G.J., 2002. Acute toxicity and cholinesterase inhibition in larval and early juvenile walleye exposed to chlorpyrifos. *Environ. Toxicol. Chem.* 21, 1469–1474.
- Polo-Cavia, N., Burraco, P., Gomez-Mestre, I., 2016. Low levels of chemical anthropogenic pollution may threaten amphibians by impairing predator recognition. *Aquat. Toxicol.* 172, 30–35.
- Pörtner, H.-O., 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J. Exp. Biol.* 213, 881–893.
- Relyea, R.A., Mills, N., 2001. Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles (*Hyla versicolor*). *Proc. Natl. Acad. Sci. U. S. A.* 98, 2491–2499.
- Rubach, M.N., Baird, D.J., Boerwinkel, M.-C., Maund, S.J., Roessink, I., Van den Brink, P.J., 2012. Species traits as predictors for intrinsic sensitivity of aquatic invertebrates to the insecticide chlorpyrifos. *Ecotoxicology* 21, 2088–2101.
- Scheil, V., Zürn, A., Köhler, H.-R., Triebeskorn, R., 2010. Embryo development, stress protein (Hsp70) responses and histopathology in zebrafish (*Danio rerio*) following exposure to nickel chloride, chlorpyrifos and binary mixtures of them. *Environ. Toxicol.* 25, 83–93.
- Schulz, R., 2004. Field studies on exposure, effects and risk mitigation on aquatic non-point-source insecticide pollution. *J. Environ. Qual.* 33, 419–448.
- Sesterhenn, T.M., Reardon, E.E., Chapman, L.J., 2013. Hypoxia and lost gills: respiratory ecology of a temperate larval damselfly. *J. Insect Physiol.* 59, 19–25.
- Shuman-Goodier, M.E., Propper, C.R., 2016. A meta-analysis synthesizing the effects of pesticides on swim speed and activity of aquatic vertebrates. *Sci. Total Environ.* 565, 758–766.
- Slos, S., De Block, M., Stoks, R., 2009. Autotomy reduces immune function and antioxidant defence. *Biol. Lett.* 5, 90–92.
- Sokolova, I.M., 2013. Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. *Integr. Comp. Biol.* 53, 597–608.
- Sokolova, I.M., Lannig, G., 2008. Interactive effects of metal pollution and temperature on metabolism in aquatic ectotherms: implications of global climate change. *Clim. Res.* 37, 181–201.
- Starostova, Z., Gvozdik, L., Kratochvil, L., 2017. An energetic perspective on tissue regeneration: the costs of tail autonomy in growing geckos. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 206, 82–86.
- Stoks, R., 1998a. Effect of lamellae autonomy on survival and foraging success of the damselfly *Lespesia sponsa* (Odonata: Lestidae). *Oecologia* 117, 443–448.
- Stoks, R., 1998b. Indirect monitoring of agonistic encounters in larvae of *Lespesia viridis* (Odonata: Lestidae) using exuviae lamellae status. *Aquat. Insects* 20, 173–180.
- Stoks, R., 1999. Autotomy shapes the trade-off between seeking cover and foraging in larval damselflies. *Behav. Ecol. Sociobiol.* 47, 70–75.
- Stoks, R., Cordoba-Aguilar, A., 2012. Evolutionary ecology of Odonata: a complex life cycle perspective. *Annu. Rev. Entomol.* 57, 249–265.
- Stoks, R., McPeek, M.A., Mitchell, J.L., 2003. Evolution of prey behavior in response to changes in predation regime: damselflies in fish and dragonfly lakes. *Evolution* 57, 574–585.
- Stoks, R., Debecker, S., Dinh Van, K., Janssens, L., 2015. Integrating ecology and evolution in aquatic toxicology: insights from damselflies. *Freshw. Sci.* 34, 1032–1039.
- Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., Erasmus, B.F.N., de Siqueira, M.F., Grainger, A., Hannah, L., Hughes, L., Huntley, B., van Jaarsveld, A.S., Midgley, G.F., Miles, L., Ortega-Huerta, M.A., Peterson, A.T., Phillips, O.L., Williams, S.E., 2004. Extinction risk from climate change. *Nature* 427, 145–148.
- Tüzin, N., Debecker, S., Op de Beeck, L., Stoks, R., 2015. Urbanisation shapes behavioural responses to a pesticide. *Aquat. Toxicol.* 163, 81–88.
- Van Buskirk, J., McCollum, S.A., 2000. Functional mechanisms of an inducible defence in tadpoles: morphology and behaviour influence mortality risk from predation. *J. Evol. Biol.* 13, 336–347.
- Verberk, W.C.E.P., Atkinson, D., 2013. Why polar gigantism and Palaeozoic gigantism are not equivalent: effects of oxygen and temperature on the body size of ectotherms. *Funct. Ecol.* 27, 1275–1285.
- Verberk, W.C.E.P., Bilton, D.T., 2011. Can oxygen set thermal limits in an insect and drive gigantism. *PLoS One* 6, e22610.
- Verberk, W.C.E.P., Bilton, D.T., 2013. Respiratory control in aquatic insects dictates their vulnerability to global warming. *Biol. Lett.* 9, 20130473.
- Verberk, W.C.E.P., Calosi, P., 2012. Oxygen limits heat tolerance and drives heat hardening in the aquatic nymphs of the gill breathing damselfly *Calopteryx vigo* (Linnaeus, 1758). *J. Therm. Biol.* (3), 224–229.
- Verberk, W.C.E.P., Bilton, D.T., Calosi, P., Spicer, J.I., 2011. Oxygen supply in aquatic ectotherms: partial pressure and solubility together explain biodiversity and size patterns. *Ecology* 92, 1565–1572.
- Verberk, W.C.E.P., Overgaard, J., Ern, R., Bayley, M., Wang, T., Boardman, L., Terblanche, J.S., 2016a. Does oxygen limit thermal tolerance in arthropods? A critical review of current evidence. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 192, 64–78.
- Verberk, W.C.E.P., Durance, I., Vaughan, I.P., Ormerod, S.J., 2016b. Field and laboratory studies reveal interacting effects of stream oxygenation and warming on aquatic ectotherms. *Glob. Chang. Biol.* 22, 1769–1778.
- Wiberg-Larsen, P., Graeber, D., Kristensen, E.A., Baattrup-Pedersen, A., Friberg, N., Rasmussen, J.J., 2016. Trait characteristics determine pyrethroid sensitivity in non-standard test species of freshwater macroinvertebrates: a reality check. *Environ. Sci. Technol.* 50, 4971–4978.
- Widder, P.D., Bidwell, J.R., 2006. Cholinesterase activity and behavior in chlorpyrifos exposed *Rana sphenocephala* tadpoles. *Environ. Toxicol. Chem.* 25, 2446–2454.
- Witt, J.W., Forkner, R.E., Kraus, R.T., 2013. Habitat heterogeneity and intraguild interactions modify distribution and injury rates in two coexisting genera of damselflies. *Freshw. Biol.* 58, 2380–2388.